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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
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| 09/972,744 | 10/05/2001 | Marcel P. Bruchez | 5100-0702 | 4380 |

23358 7590 01/30/2007
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| EXAMINER | |
|---|---|
| DO, PENSEE T | |
| ART UNIT | PAPER NUMBER |
| 1641 | |

| SHORTENED STATUTORY PERIOD OF RESPONSE | MAIL DATE | DELIVERY MODE |
|---|---|---|
| 3 MONTHS | 01/30/2007 | PAPER |

**Please find below and/or attached an Office communication concerning this application or proceeding.**

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 09/972,744 | BRUCHEZ ET AL. | |
| | Examiner | Art Unit | |
| | Pensee T. Do | 1641 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *10 November 2006*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *1-6,11-14,16-20,25-35,38 and 74-79* is/are pending in the application.

4a) Of the above claim(s) *16-20,25-35 and 38* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-6,11-14 and 74-79* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☒ Claim(s) *1-6,11-14,16-20,25-35,38 and 74-79* are subject to restriction and/or election requirement.

**Application Papers**

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *11/10/06*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

## DETAILED ACTION

### *Amendment Entry & Claims Status*

The amendment filed on November 10, 2006 has been acknowledged and entered.

Claims 1-6, 11-14, 16-20, 25-35, 38, 74-79 are pending.

Claims 16-20, 25-35, 38 are withdrawn from further consideration.

Claims 1-6, 11-14, 74-79 are being examined.

### *Claim Rejections - 35 USC § 103*

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-6, 11-14, 74, 75 are rejected under 35 U.S.C. 103(a) as being unpatentable over Millard et al. (US 5,534,416) in view of Bawendi et al. (US 6,306,610).

Millard teaches a cell encoded with two different fluorescent dyes, dye 1 and dye 2. Dye 1 is highly membrane permeant and labels all cells. Dye 1 binds with intracellular nucleic acid polymers such as DNA and RNA and the resultant cell becomes extremely fluorescent upon illumination. (see col. 3, line 65-col. 4, line 18). Dye 2, which is detectably different than dye 1, stains the viable cells and such labeled cells give a fluorescent response inside the viable cells. (see col. 13, lines 60-65).

However, Millard fails to teach labeling the cell with a semiconductor nanocrystal; the detectable label comprises a semiconductor nanocrystal conjugated to a translocatable molecule which is a ligand for a cellular receptor that enters the cell by endocytosis.

Bawendi teaches a composition comprising fluorescent semiconductor nanocrystals associated to a molecule such as cells, prokaryotic or eukaryotic. The semiconductor nanocrystals comprise a CdSe core and a ZnS shell. The composition is also associated with cell membranes. (see col. 3, line 60-col. 4, line 62; col. 19, lines 58-60; col. 20, lines 51-59; col. 29, lines 41-42). Bawendi also teaches nanocrystals coupled to antibodies to a cellular target/component. (col. 5, lines 10-17).

It would have been obvious to one of ordinary skills in the art to replace one of the fluorescent dyes in Millard with semiconductor nanocrystals of Bawendi because nanocrystals exhibit high fluorescent intensity (for detection in small quantities), a separation of at least 50 nm between the absorption and fluorescing frequencies, solubility in water, ability to be readily linked to other molecules, stability towards harsh conditions and high temperatures, symmetric, nearly Gaussian emission lineshape for easy deconvolution of multiple colors and compatibility with automated analysis. Regarding claims 4-5, Millard (see col. 3, line 1) and Bawendi teach prokaryotic and eukaryotic cells (see col. 19, line 65), it would have been obvious to one of ordinary skills in the art to experiment cells such as yeast, mammalian cells of rat, mouse, bovine, hamster, and plant cell since it is well known that those cells are eukaryotic cells. Regarding claim 75, since Bawendi teaches that the nanocrystal can couple to a

reagent for detection of biological compounds, organelles and studying endocytosis, it would have been obvious to one of ordinary skills in the art that such "reagent for detection of biological compounds, organelles" is a ligand for a cellular receptor that enters the cell by endocytosis because in order to study endocytosis, the cells must be labeled with the nanocrystal which in turn must be coupled to a ligand or a molecule that enters the cell membrane. It is well known that "endocytosis" is one of the methods for a compound to translocate across the cell membrane.

Claim 77 is rejected under 35 U.S.C. 103(a) as being unpatentable over Milllard in view of Bawendi et al. (US 6,306,610) as applied to claim 1, and further in view of Rothbard et al. (US 6,306,993).

Millard and Bawendi have been discussed above. In addition, Millard and Bawendi teach that the fluorescence dyes or semiconductor nanocrystals can associate with a molecule or reagent for detection of a biological compounds such as enzymes, DNA, RNA, cellular organelles, cell membrane molecules involved in signal transduction and such composition can be used to detect cell morphology and fluid flow, cell viability, proliferation and function; endocytosis and exocytosis. (see col. 20, lines 50-60).

However, Millard and Bawendi fail to teach the nanoparticle is conjugated to a ligand for a transporter.

Rothbard teaches methods and composition for transporting drugs and macromolecules across biological membranes wherein the biological membranes are contacted with a conjugate containing a biologically active agent (ligand) that is

covalently attached to a transport polymer (transporter). Such transport polymer has 6 to 25 subunits of L-Arginine. The transport enhancing polymers are exemplified by peptides in which arginine residues constitute the subunits. Exemplary eukaryotic cell membranes of interest include membranes of dendritic cells, epithelial cells, endothelial cells, keratinocytes, muscle cells, fungal cells, bacterial cells, plant cells and the like. Biological active agents are macromolecules such as nucleic acids, peptides, proteins and analogs thereof. The agent may be linked to the polymer by a linking moiety. The composition includes a conjugate containing a biological active agent covalently attached to at least one transport polymer and can be packaged with instructions for using it. (see col. 2, line 44-col. 4, line 45; col. 5, lines 47-58). The transport polymers contain short-length polymers from 6 to 25 subunits. The conjugate is effective to enhance the transport rate of the conjugate across the biological membrane relative to the transport rate of the non-conjugate biological agent alone. (see col. 6, line 63-col. 7, line 5). Detecting uptake of macromolecules may be facilitated by attaching a fluorescent tag. (see col. 11, lines 3-4). Fluorescently labeled peptide polymers composed of 6 or more arginine residues entered cells more efficiently than the tat sequence 49-57 in fig. 1 (see col. 11, lines 30-40). Since the polymer of Rothbard composes of 6 to 25 contiguous Arg residues, it must be a cationic polymer.

Since Millard, Bawendi and Rothbard teach using a label such as nanocrystals and fluorescent dyes for cells or cell membrane, it would have been obvious to one of ordinary skills in the art to associate the polymer composition (comprising a ligand coupled to a transporter) taught by Rothbard to the nanocrystals as a fluorescent label

and use in the combined composition taught by Millard and Bawendi because macromolecules such as peptides and oligonucleotides experience difficulty in passing across the biological membrane and having a polymer as a transportable molecule as that of Rothbard enhances trans-membrane transport. Furthermore, the nanocrystals of Bawendi can be used as a label that associates with the polymer to so that measures of biological molecules transported across the biological membrane can be easily detected because the nanocrystals of Bawendi associates with the biological membrane.

Claim 76 is rejected under 35 U.S.C. 103(a) as being unpatentable over Millard in view of Bawendi as applied to claim 1, and further in view of Rothbard as applied to claim 74 above, and further in view of Sodroski et al. (US 6,761,902).

Millard, Bawendi and Rothbard have been discussed above.

However, these references fail to teach that the translocatable molecule is a ligand for a G-protein coupled receptor (GPCR).

Sodroski teaches that G-protein coupled receptors (GPCR), which span the membrane seven times. These are functionally linked to signaling proteins known as G-proteins. (see col. 8, lines 13-52).

Therefore, it would have been obvious to one of ordinary skills in the art to couple the transmembrane receptor GPCR as taught by Sodroski to the "reagent for detecting biological compound" to study the endocytosis and exocytosis or to encode the cell as taught by Millard, Bawendi and Rothbard because the transmembrane receptor GPCR can cross the cell membrane seven times. Thus, study of endocytosis or exocytosis can be effectively performed.

Claim 78 is rejected under 35 U.S.C. 103(a) as being unpatentable over Millard in view of Bawendi et al. (US 6,306,610) as applied to claim 1, and further in view of Frankel et al. (US 5,652, 152).

Millard and Bawendi have been discussed above.

However, Millard and Bawendi fail to teach that the translocatable molecule is a HIV-Tat protein.

Frankel teaches intracellular delivery of cargo molecules by the use of transport polypeptides which comprise HIV tat protein or one or more portions thereof and which are covalently attached to the cargo molecules. The transport polypeptides are characterized by the presence of the tat basic region (amino acids 49-57). The biological active cargo molecules such as polypeptides, nucleic acids are delivered/transported into the cytoplasm and nuclei of cells in vitro and in vivo. (see abstract). Label such as a fluorescent was used to study the transported molecules across the cell membrane. The label is attached to the tat peptide. (see col. 42, lines 24-29).

It would have been obvious to one of ordinary skills in the art to use the HIV tat peptide for transporting biological molecules across the cell membrane as taught by Frankel and attach it to a fluorescence semiconductor nanocrystal which associates to a cell membrane or a subcellular organelle so that when biological molecules to be transported reach the cell membrane, they can be transported effectively and efficiently with the aid of the tat peptide and their activity or measurement can be detected by the nanocrystals since the nanocrystals have a spectral emission that is tunable to a

desired wavelength, and wherein said wavelength provides information about a biological state or event.

Claim 79 is rejected under 35 U.S.C. 103(a) as being unpatentable over Millard in view of Bawendi as applied to claim 1, and further in view of Barbera-Guillem (US 6,194,213).

Millard and Bawendi have been discussed above.

However, Millard and Bawendi fail to teach the composition further comprises a liposome.

Barbera-Guillem teaches a composition comprising functionalized nanocrystals and lipid membrane labeled such nanocrystals. Lipid membranes include cell membranes, liposomes, and lipid membrane-coated biosensors. (see col. 3, line 55-col. 4, line 20).

It would have been obvious to one of ordinary skills in the art to use liposomes as a lipid membrane as taught by Barbera-Guillem in the combined composition of Millard and Bawendi since these references teach using nanocrystal and fluorescent dye to label lipid membrane or cell membrane. Liposome is well known as a carrier or delivery vehicle for drugs, proteins, or other compounds. Thus, having a liposome as part of the composition of Bawendi can transport the nanoparticles across the cell membrane.

***Response to Arguments***

Applicant's arguments filed November 10, 2006 have been fully considered but they are not persuasive.

Regarding the 103 rejection for claims 1-6, 11-14, 74 and 75, 79, Applicants argue that: a) the restriction requirement, between the method of distinguishing and identifying a cell and the composition comprising a cell encoded with a nanocrystal and an organic fluorophore based on the reason that the method can be practiced with another materially different product such as magnetic particles or dye to label cells, renders the dye labeled cells patentably distinct and non-obvious from nanocrystal labeled cells. Thus, the proposition of replacing the nanocrystal of Bawendi with one of the fluorescent dyes in Millard is contradictory to the initial finding that the nanocrystal is not obvious in view of a dye labeled cell.

b) Nanocrystals are not obvious replacements for the dyes in Millard: because nanocrystals exhibit poor solubility, high rigidity, core hydrophobicity, large particle diameter and high ionic density that can adversely affect incorporation, mobility, and subsequent viability of live cells. This is contrasted with small molecules dyes, which are small, dynamic, generally hydrophilic and have limited ionic charges allowing them to passively enter , move and not affect viability of live cells. Millard is directed to the detection of cell viability in a sample. Accordingly, it is unlikely that one would be motivated to replace a large, bulky, ionic sphere or semiconductor nanocrystal as described in Bawendi, with the easily transported inert dyes described in Millard. Therefore, nanocrystals are not an obvious replacement for dyes, such as those described by Millard.

Response:

a) the restriction requirement is between the method identifying cells and a composition comprising cells encoded with nanocrystals. The method can be practiced with a different product, of either magnetic particles or dye to label cells. The restriction is still valid because the method can be practiced with magnetic particles, if not with dye. However, it does not mean that the dye is non-obvious from the nanocrystals because the restriction was not between a dye labeled cell and a nanocrystal labeled cell.

b) Applicants' argument about how the characteristics, of the nanocrystals , which can adversely affect incorporation, mobility and viability of cells is an admission that the claims as recited are unable. Bawendi teaches that nanocrystals can be associated with a molecule for detection of a biological compounds such as enzymes, RNA, DNA, cellular organelles, etc., detection of cell viability, endocytosis, exocytosis.(see col. 20, lines 50-60). Therefore, the nanocrystals of Bawendi are able to penetrate the cells, and live cells in order to detect cellular organelles and cell viability. Furthermore, Bawendi teaches the advantages of using nanocrystals set forth in the previous office action. Thus, the statement about nanocrystals adverse affecting the incorporation, mobility, and viability of cells is merely just an assertion without any documentation and is contradictory to the teaching of Bawendi that nanocrystals can be used to detect cellular organelles, which are located within the cell, and cell viability.

Regarding the rejection of claim 77, which recites that the translocatable molecule is a ***ligand*** for a transporter. Rothbard teaches an biological active agent for binding to a transport polymer (transporter). The claim only requires that the

translocatable is a ligand for a transporter, such translocatable molecule is conjugated to nanocrystals, and nothing else. However, Applicants argue that such transporter in Rothbard is only good for transporting small molecules, but not large and bulky molecules such as nanocrystals. It is found that Applicants' argument is irrelevant to what is being claimed. The biological active agent in Rothbard is a defined to be a protein, peptide, etc. (see col. 5, lines 59-64). These biological active agents can bind to a transporter polymer. It is taught by Bawendi that these biological active agents can be coupled to nanocrystals. Thus, one of ordinary skills in the art would have no problem conjugating the nanocrystals to proteins and peptides, which can be bind to a transport polymer, regardless of how the translocatable or the transporter is being used.

Applicants use the same arguments for claim 76, therefore, no further discussion is necessary.

Regarding claim 78, Applicants argue that, similar to Rothbard, the "cargo" molecules in Frankel are biological molecules (peptides, nucleic acids, oligonucleotides), not nanocrystals. The biological molecules are smaller compared to the nanocrystals and thus can enter the cells more readily than nanocrystals.

Bawendi teaches that nanocrystals can be conjugated with biological molecules, such as proteins, peptides, etc. (see col. 7-8). Thus, one of ordinary skills in the art would have reasonable expectation of success in conjugating the nanocrystals of Bawendi with the HIV tat peptide of Frankel or replacing the fluorescent label of Frankel with the nanocrystals because of the advantages of the using nanocrystals as set forth in Bawendi, regardless of how the translocatable or the transporter is being used.

***Conclusion***

**THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Pensee T. Do whose telephone number is 571-272-0819. The examiner can normally be reached on Monday-Friday, 7:00-3:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Long Le can be reached on 571-272-0823. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Pensee T. Do
Patent Examiner
January 16, 2007

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